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Rhodolith primary and carbonate production in a changing ocean: The interplay of warming and nutrients

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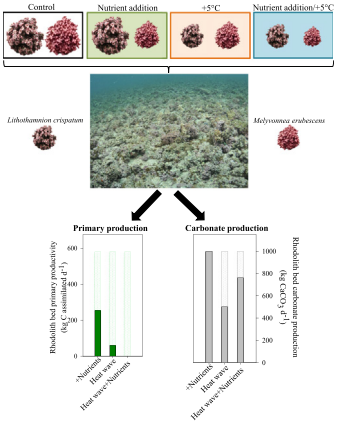
HIGHLIGHTS

- Rhodolith response to increased temperature and nutrient concentration were studied.
- Stressors affected rhodolith physiological performance negatively.
- No interactive effects were found between stressors.
- Species-specific responses-severity of impact depends on community composition.
- Heat waves will affect rhodolith beds, and even more so in nutrient-rich regions.

GRAPHICAL ABSTRACT



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ABSTRACT

Rhodolith beds, like many other marine ecosystems, are affected by climate change that is causing an increase in the magnitude and frequency of extreme high temperature events (heat waves). Unfortunately, this does not represent the sole peril for these communities, as coastal urbanization in conjunction with altered precipitation patterns can increase terrestrial-derived nutrient input. In Brazil, rhodolith beds are among the most extensive coastal benthic ecosystems, but despite their vast distribution and great ecological and economic importance, studies on the productivity of these communities and the impact of changing environmental conditions are almost non-existent. This study addressed the individual and combined effects of increases in temperature and nutrient concentration on the physiological performance of two widely distributed rhodolith species, *Lithothamnion*

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crispatum and *Melyvonnea erubescens*. The results showed species-specific responses in net photosynthetic performance, with no response in *L. crispatum*, while *M. erubescens* responded negatively to both increase in temperature and nutrients. In contrast, calcification in both species showed a significant decline at high temperature. No interactive effects were found between temperature and nutrients, yet their combined negative effects were additive, resulting in negative daily-integrated net productivity and a large decline in daily carbonate production in both species. This has strong implications for rhodolith bed primary productivity and carbonate production, as heat waves may potentially cause a strong decline in carbonate production (ca. 50% loss), accompanied by a severe drop in primary productivity that will be even more pronounced under high-nutrient conditions. Also, the species-specific responses to changes in temperature and nutrient concentration suggest that the magnitude of impact of these factors on rhodolith bed productivity will depend on the species dominating the community and may finally result in changes in rhodolith community composition.

1. Introduction

Rhodolith beds, composed of free-living coralline algae, are among the most important benthic communities, as they cover vast coastal areas throughout polar, temperate, subtropical and tropical shallow seas (Foster, 2001; Riosmena-Rodríguez et al., 2017). They build environments recognized as hot-spots of biodiversity that hold very high population densities, as both their living and dead thalli produce three-dimensional structures that provide ecological niches for a diverse range of seaweeds and invertebrate species (e.g. Kamenos et al., 2004; Grall et al., 2006; Amado-Filho et al., 2007, 2010; Riul et al., 2009). In addition, they contribute significantly to the global carbon cycle, functioning as major natural factories and storage grounds of biogenic $CaCO_3$ sediments. Recent empirical estimations suggest that the marine carbonate deposits generated by coralline algae represent a total potential carbon sink of 4×10^8 t C yr^{-1} (van der Heijden and Kamenos, 2015), indicating that rhodolith beds may represent an underappreciated significant carbon store (Hill et al., 2015; Macreadie et al., 2017).

Rhodolith beds, like many other marine ecosystems, are threatened by altered environmental conditions associated with ongoing global climate change, such as increasing seawater temperatures, ocean acidification and sea level rise. These phenomena, coupled with increasing rainfall and stronger storm events, increase sedimentation and turbidity in coastal areas (resuspended sediments, increased terrestrial run-off) (IPCC, 2014). The potential impacts of those changes can be separated into those caused by gradual changes in climatic conditions ("trends") and those produced by changes in the magnitude and/or frequency of extreme events ("events") (Easterling et al., 2000; Jentsch et al., 2007). In this context, mounting evidence over recent years has shown that, in conjunction with gradual warming trends, discrete events of anomalous high seawater temperature (marine heatwaves, MHWs) are becoming more frequent and intense (e.g., Meehl and Tebaldi, 2004; Frölicher et al., 2018; Oliver et al., 2018). These events have already caused severe impacts on different marine benthic ecosystems (e.g., Garrabou et al., 2009; Marbá and Duarte, 2010; Wernberg et al., 2013; Short et al., 2015), contributing to the growing consensus of the importance of understanding ecological responses to "events" rather than "trends", as climate extremes can drive profound ecological changes over short-time scales (Jentsch et al., 2007, 2011; Thompson et al., 2013; Ghedini et al., 2015; Smale et al., 2015).

The potential of extreme temperature events to impact marine ecosystems is not surprising, as temperature is probably the most important rate-determining factor in biology, through its impact on species physiology (metabolism, growth, reproduction, etc.) (Eggert, 2012). In coralline algae it has been shown to be a major driver for their geographical distribution (Wilson et al., 2004) and the temporal and spatial variability of their calcification rates (Blake and Maggs, 2003; Martin et al., 2006; Kamenos and Law, 2010; Adey et al., 2013). Experimental studies on coralline algae have shown general promoting effects of increased temperature on species' metabolism but also revealed a high sensitivity of the algae when these increases exceed the local summer temperature maxima (reviewed in Martin and Hall-Spencer, 2017). Thus, episodes of suddenly anomalous high temperatures can have strong effects on coralline algae, as observed, for example, with their high mortality in Western Australia during a heatwave event (Short et al., 2015).

Besides temperature-associated impacts, climate change-related alteration of precipitation patterns can influence terrestrial-derived nutrient delivery to the coastal seas, via changes in river flow. This, together with the existing local impacts on rhodolith beds due to increasing coastal urbanization and human activities (i.e., aquaculture, agricultural run-off, eutrophication, and sewage) (Barbera et al., 2003; Grall and Hall-Spencer, 2003; Hall-Spencer et al., 2006), will further affect coastal rhodolith communities. The effects can be direct, due to nutrient effects on rhodolith performance (Björk et al., 1995; Shayka, 2018), or indirect, through increased sedimentation and phytoplankton growth, leading to decreased light availability (Hall-Spencer and Moore, 2000; Wilson et al., 2004; Riul et al., 2008; Halfar et al., 2012; Villas-Boas et al., 2014). In addition, the effect of both increasing seawater temperature and local stressors (e.g. nutrient loading) might be underestimated if there are interactive effects (especially synergistic effects, see Brown et al., 2013) that can magnify the impacts (e.g., Russell et al., 2009). Though, some rhodolith communities may present higher resilience to the aforementioned factors, e.g. the recently discovered rhodolith beds at the Amazon River mouth (Moura et al., 2016; Vale et al., 2018).

In light of these threats, assessing the impacts of global and local stressors and their potential interactions on keystone species of rhodolith communities is of great importance. For this purpose, the use of physiological approaches is particularly useful, as any effect at this level will translate into cascading ecological effects in the organisms (i.e., growth, abundance, and distribution) and the associated fauna and flora. Unfortunately, studies on rhodolith physiology and their responses to environmental changes, such as those related to climate change, are few and mostly focused on temperate European species (e.g., Martin et al., 2006; Noisette et al., 2013a, 2013b; Legrand et al., 2017; Sordo et al., 2018). Studies on the direct effects of nutrient enrichment on rhodolith performance, either as an isolated factor or in combination with changes in other factors, are almost non-existent.

Along the Brazilian coast, rhodolith beds extend almost continuously over the continental shelf from 2°N to 23°S, with the southernmost bed located at Arvoredo Island (27°S) (reviewed in Amado-Filho et al., 2017). With an estimated 2×10^{11} tons of $CaCO_3$, these beds are a major reservoir of carbonate deposits (Milliman, 1974). So far, extensive sampling efforts have allowed a better understanding of rhodolith bed distribution and species composition along the Brazilian coast, considering the beds in a biological context (reviewed in Horta et al., 2016; Amado-Filho et al., 2017). Yet, to date there is still a considerable lack of physiological data from Brazilian rhodolith species in general (Riul et al., 2008; Figueiredo et al., 2012; Cavalcanti et al., 2014) and in particular on how these algae might respond to both global and local stressors (Horta et al., 2016). In the present study we investigated the isolated and

combined effects of a sudden temperature increase (i.e., heat wave event) and increased nutrient concentration on the physiological performance of two of the most widely distributed Brazilian rhodolith species, *Lithothamnion crispatum* Hauck, 1878 and *Melyvonnea erubescens* (Foslie) Athanasiadis & D.L. Ballantine, 2014 (Amado-Filho et al., 2017). In addition, using existent data on species' densities and rhodolith bed extension, we estimated the impacts of different scenarios of temperature and increased nutrients on the productivity and carbonate production of the studied subtropical rhodolith bed.

2. Material and methods

2.1. Study area and sample collection

The Arvoredo Reserve (Fig. 1a) is a "no take" marine protected area in the Southwestern Atlantic coast, approximately 6.5 km east of the coast of the state of Santa Catarina, Brazil. It is located in a highly dynamic region, influenced both by cold, nutrient rich waters from the La Plata River and Falkland current (during winter) and by warm coastal waters from the Brazil current during summer (Piola et al., 2005; Matano et al., 2010). Thus, the temperature in this region exhibits a well-defined seasonal pattern, with recorded mean seawater temperature at Rancho Norte between 17 and 27 °C in winter and summer, respectively (Fig. 1b; Sarti and Segal, 2018). Light availability also varies seasonally, with maximum depths of the euphotic zone ranging from 59 m (summer) to 30 m (winter), corresponding to a K_d of 0.08 and 0.16 m^{-1}, respectively (MAArE, 2017). Recently, an increase in the frequency and intensity of heat wave events (sensu Hobday et al., 2016) has been reported for the region, with recorded sudden temperature increases of up to 4 °C and durations of up to 41 days, strongly impacting coastal phytobenthic communities (Gouvêa et al., 2017). In addition, the region is affected by frequent upwelling events, urban and agriculture run-offs, and by the La Plata River plume (Piola et al., 2005), all of which increase nutrient concentrations (Pagliosa et al., 2006).

The rhodolith bed at Rancho Norte, within the Arvoredo Marine Biological Reserve, Brazil (−27°16′25.8″, −48°22′0.99″), extends from 6 to 20 m depth and is composed of six rhodolith species, of which *Melyvonnea erubescens* (fruticose, with branched protuberances) and *Lithothamnion crispatum* (lumpy to fruticose, with cylindrical protuberances flaring distally into curly margined, funnel-like or cup-shaped tips) dominate the community (Fig. 1c–e; Metri, 2006; Pascelli et al., 2013; Carvalho, 2018).

Rhodolith samples were collected in May 2017 (Austral autumn) from ~8 m depth by SCUBA diving. Immediately after collection, samples kept in coolers with seawater were transported to the mesocosm facility at the Federal University of Santa Catarina and transferred to tanks (V = 100 L) with circulating seawater, which passed through sand filters of different pore sizes (25, 20 and 5 μm) and were exposed to UV light before entering the mesocosm system. The rhodoliths were pre-acclimated for two weeks under natural light conditions, adjusted to the mean maximum light level at collection depth (K_d = 0.12 m^{-1}; ~41% of incident light), and 23 °C, the temperature in the field during sample collection. Before the beginning of the experiment, the temperature was increased by 1 °C per day until reaching 28 °C in the respective high-temperature treatment tanks.

2.2. Experimental design

A bi-factorial experiment was conducted for 30 days under natural light conditions, exposing the rhodolith samples to four treatments across two variables: temperature (23 °C and 28 °C) and nutrients (low and high nutrient levels).

The experimental set-up consisted of eight reservoir tanks (four at 23 °C, four at 28 °C), which were used as temperature-controlled water baths; in turn, each reservoir tank supplied water to four smaller tanks (Fig. 2). Temperature was controlled in the reservoirs by aquarium heaters and chillers (Radical 1 HP, Brazil). Inside each smaller tank, the samples (n = 5 rhodoliths) were held in a 1.5 L container fitted with a small submersible pump to ensure water circulation. In each

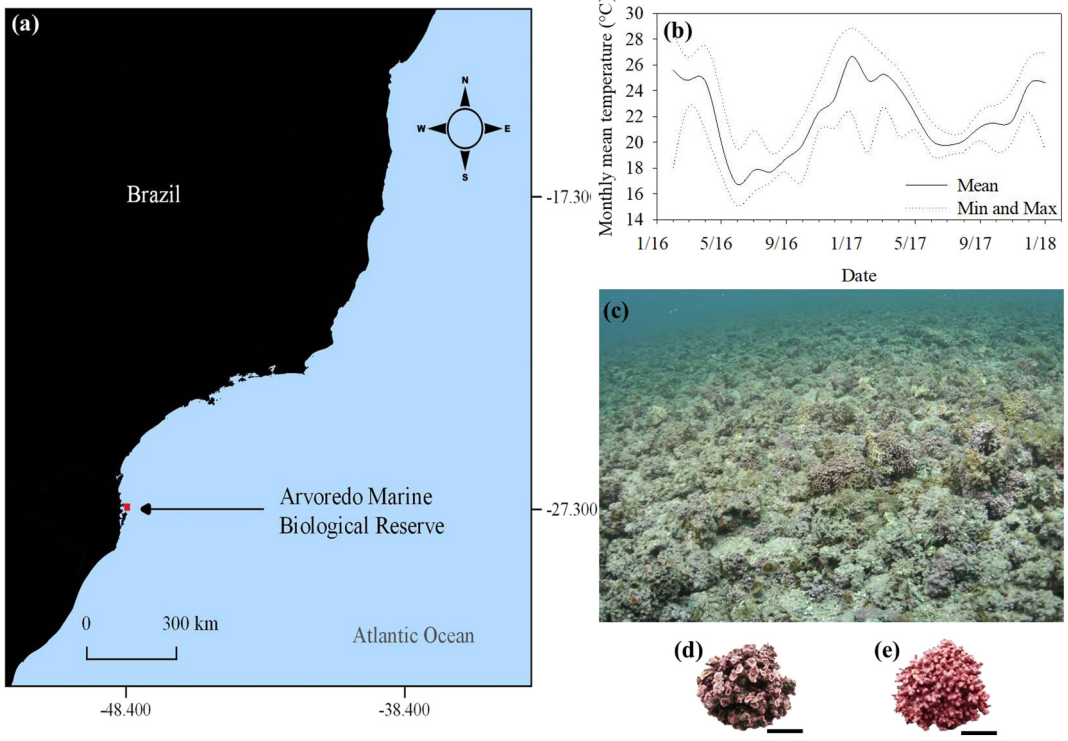

Fig. 1. (a) Sampling location at Arvoredo Island, Brazil, (b) the monthly mean seawater temperature at the location (recorded at 10 m depth; Sarti and Segal, 2018) and (c) the rhodolith bed at Rancho Norte, Arvoredo Island, dominated by the studied species (d) *L. crispatum* and (e) *M. erubescens* (Scale: 2 cm). Photos by Amanda da Silva Góes and João Silva.

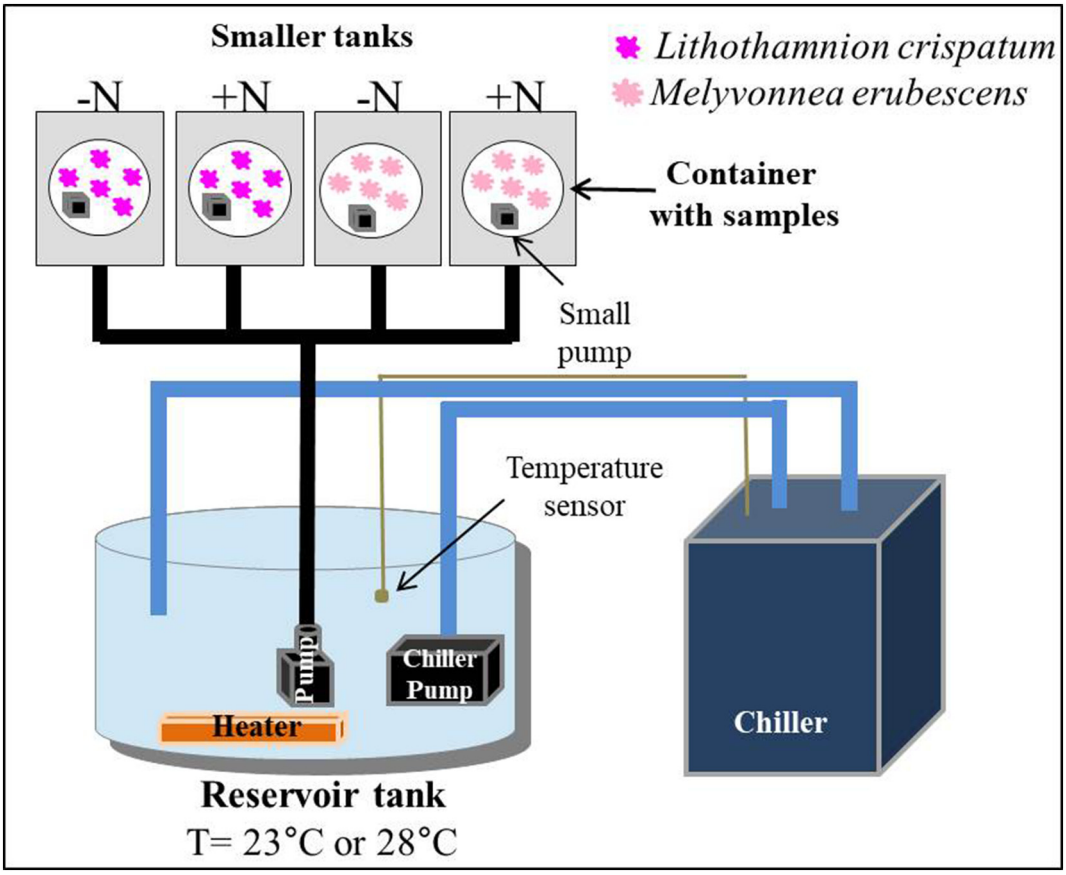

Fig. 2. Schematic representation of the experimental set-up, showing one experimental unit (−N – without nutrient addition, +N – with nutrient addition). Water was circulated through the chiller via a chiller pump and a heater was placed within each reservoir tank. Another pump circulated the water through four smaller tanks which served as water baths, keeping all tanks at constant temperature. Each smaller tank held a container immersed in the water bath, which housed the rhodoliths. This set-up allowed for different nutrient treatments to be tested within the same temperature treatment conditions (i.e., each container with rhodoliths contained its own seawater and is an independent statistical unit). Each temperature treatment included four experimental units.

temperature-controlled unit (Fig. 2) there were two containers per species, one to which nutrients were added (+N) and one without nutrient addition (−N), thus giving an n = 4 per treatment and species. During the experiment, water temperature and irradiance were monitored with HOBO Pendant Temperature/Light Data Loggers (Onset, Bourne, USA). The recorded mean maximum irradiance during the experiment was ~300 μmol quanta m^{-2} s^{-1} (ranging between 144 and 567 μmol quanta m^{-2} s^{-1}), yielding a mean daily integrated light exposure of 5.3 mol m^{-2} (ranging between 1.4 and 9.8 mol m^{-2}).

Seawater inside the containers was changed every two days, after which nutrients were freshly added in the (+N) treatments. At the beginning of the experiment, samples for nutrient analyses were taken from the different treatments before and after nutrient addition, showing that ambient nutrient concentrations in the seawater (without nutrient addition) were: 2.0 μM NO_3, 1.1 μM NH_4, 1.6 μM PO_4. To these levels, 21 μM NO_3, 70 μM NH_4 and 4 μM PO_4 were added to simulate increased nutrient availability. These concentrations were chosen as they represent reported mean nutrient concentrations in the urbanized areas of Santa Catarina Island (Pagliosa et al., 2006).

At the end of the experiment, rhodoliths were carefully cleaned with a toothbrush used for separate analyses: two rhodoliths as sub-replicates for photosynthesis, calcification, and C:N content, two rhodoliths for enzyme activity and one rhodolith for pigment content.

2.3. Photosynthesis, respiration and light calcification measurements

Physiological responses to the different treatments were measured after 30 days of exposure on two sub-replicate samples from each container (n = 4 replicates per treatment and species). Photosynthetic O_2 evolution measurements were carried out using laboratory incubations in sealed custom-made acrylic chambers (V = 150 mL) with internal circulation provided by a magnetic stirrer. The rhodoliths were incubated with filtered seawater (0.45 μm) from the respective treatment and at the respective treatment temperature for 1 h at 300 μmol quanta m^{-2} s^{-1}. The oxygen concentration was measured at the beginning and the end of the incubations with an oxymeter (YSI 5000-115, Yellow Springs, USA) and water samples were taken before and after the light incubations, poisoned with $HgCl_2$ and stored in borosilicate tubes (two tubes per incubation chamber, V = 12 mL each) for later estimation of light calcification rates. Afterwards, the rhodoliths were incubated in darkness for 30 min to determine respiration rates, which allowed for later calculation of gross photosynthesis. Later, the rhodoliths were dried for 48 h at 60 °C and the estimated dry weight was used to normalize metabolic and calcification rates. The dried samples were subsequently used for determination of carbon and nitrogen tissue contents (see below).

The calcification rates of the rhodolith species were determined from alkalinity measurements of samples before and after incubation, using the alkalinity anomaly principle based on the ratio of two equivalents of total alkalinity per each mole of $CaCO_3$ precipitation (Smith and Kinsey, 1978). For alkalinity measurements, triplicate analyses of each sample were performed, using the spectrophotometer procedure of Yao and Byrne (1998). Water samples were gently bubbled with N_2 for at least 10 min, 3 mL transferred into a 1 cm optical-path length polystyrene cuvette, to which 10 μL of Bromocresol Green (BCG; Sigma Aldrich, Steinheim, Germany) were added. Microtitration with

0.3 N HCl was carried out at a rate of 8 μL min^{-1}, using a glass syringe (Hamilton Company, Reno, USA) operated by a syringe pump (Kd Scientific Inc., Holliston, USA). During titration, changes in the absorbance at 444, 616 and 750 nm were continuously recorded with an Ocean Optics USB2000 spectrophotometer (Ocean Optics, Dunedin, USA), using a tungsten halogen light source (HL-2000, Ocean Optics, Dunedin, USA). For quality control, a certified reference material of known total alkalinity was used to calibrate the method (Batch 129; supplied by the Marine Physical Laboratory, Scripps Institution of Oceanography, USA).

2.4. Pigment analysis

The hydrophilic (phycobilipigments) and lipophilic pigments (chlorophyll *a*) were determined by a two-step extraction, according to Kursar et al. (1983), with slight modifications. Phycobilipigment extraction was done by grinding the algal tissue (n = 4 replicates per treatment and species) with liquid nitrogen and extracting with potassium phosphate buffer (0.1 mol L^{-1}, pH 6.8) at 4 °C for 2 h in darkness. Subsequently, the extract was centrifuged (3500 rpm, 2 min), and phycobilipigment content was determined spectrophotometrically in the supernatant. The pellet was resuspended with 90% acetone and kept overnight in darkness at 4 °C, to extract chlorophyll *a*. The phycobilipigment content was calculated according to Kursar and Alberte (1983), and chlorophyll *a* was calculated using the equation of Porra et al. (1989). The pigment concentrations were normalized by algal fresh weight.

2.5. Nitrate reductase activity (NRA)

The activity of the enzyme nitrate reductase was measured in situ, using the methodology of Corzo and Niell (1991). Living rhodoliths (n = 2 subreplicates per container, n = 4 replicates per treatment and species) were incubated for 1 h with 10 mL of assay buffer (0.1 M phosphate buffer containing 0.5 mM EDTA, 0.1% 1-propanol, 30 mM KNO_3, 10 μM glucose) in darkness at 30 °C. To prevent O_2 in the assay medium, the assay buffer was bubbled with N_2 for 2 min immediately before samples were inserted, and then again for another 2 min. After incubation, the reaction was stopped by removing 2 mL of the assay buffer, to which 400 μL of 4% sulphanilamide and 400 μL of 0.1% n-(1-napthyl) ethylenediamine dihydrochloride were added. The nitrite concentration was measured spectrophotometrically at 543 nm (Snell and Snell, 1949), after a 30 min period to allow for complete color development, and the concentrations of NO_2^- in the samples were calculated based on a calibration curve using $NaNO_2$. Following the assay, the rhodoliths were dried at 60 °C for 48 h, weighed and NRA was calculated as μmol NO_2 g DW^{-1} h^{-1}.

2.6. Carbon and nitrogen content

One of the oven-dried samples, used previously for photosynthesis and calcification determinations, were ground with mortar and pestle and afterwards, a subsample was weighted into a tin capsule (n = 4, 1–2 mg dry weight each). Carbon and nitrogen contents were determined through elemental analysis, using a Vario EL III elemental analyser (Elementar).

2.7. Water nutrient analysis

Seawater samples were passed through GF/F Whatman 0.45 μm filters before analysis. The dissolved inorganic nutrients (NO_3^{-2}, NH_4^+, PO_4^{-3}) were determined colorimetrically, according to Tréguer and Le Corre (1975) and Grasshoff et al. (1983), using a spectrophotometer (UV-1100, Pró-Análise ISE Química e Diagnóstica Ltda, Brazil).

2.8. A posteriori estimations of daily-integrated rhodolith primary and carbonate production

To estimate daily rhodolith primary production in terms of carbon fluxes, oxygen produced by photosynthesis or consumed by respiration was converted into carbon equivalents, using the photosynthetic (PQ = mol O_2 fixed/mol C produced) and respiratory quotient (RQ = mol C produced/mol O_2 fixed) estimated previously for rhodoliths (PQ = 1.17, RQ = 0.97; Martin et al., 2006). Then, daily productivity budgets were calculated from the measured gross photosynthetic, respiratory and calcification rates, considering a daily cycle of 12 h light:12 h dark. Night calcification totals were calculated using previously determined dark calcification rates of 20.1% and −33% of maximum light calcification rates for *L. crispatum* and *M. erubescens*, respectively (Schubert, unpublished data).

For extrapolation purposes to the rhodolith bed at Arvoredo Island, where the samples were collected, reported densities of the studied species per square meter bed were used: 4658 g DW m^{-2} for *L. crispatum* and 4878 g DW m^{-2} for *M. erubescens* (Carvalho, 2018). This, together with the reported extension of the rhodolith bed in the Marine Biological Reserve of Arvoredo (100,000 m^2; Gherardi, 2004) allowed an estimate of the rhodolith bed productivity and carbonate production, as well as of the potential impacts of different scenarios, including increased nutrient concentrations and heat wave events.

2.9. Statistical analyses

Statistical analyses were performed using the software STATISTICA 7.0 (StatSoft, Inc., U.S.A.). Interactive and isolated effects of temperature and nutrient concentrations were evaluated for each species and for all measured parameters, using two-way ANOVAs. To determine species-specific responses to temperature and nutrient concentration in daily-integrated rhodolith bed primary and carbonate production, three-way ANOVA was used, with species, temperature and nutrient concentration as factors. Also, to test for differences among species in primary and carbonate production per m^2 of rhodolith bed, one-way ANOVA was used. Newman-Keuls Significant Difference post-hoc tests were used to identify the statistically different groups. Data were tested a priori for normality, using the Shapiro-Wilk test.

3. Results

3.1. Temperature and nutrient effects on rhodolith primary and $CaCO_3$ production

The rhodolith photosynthetic performance showed species-specific differences. Both gross photosynthesis and respiration of *L. crispatum* increased significantly with temperature by a factor of 1.2 and 2–2.5, respectively, independent of nutrient concentration, resulting in similar net photosynthesis in all treatments (Fig. 3a; Table 1). In contrast, *M. erubescens* exhibited a significant reduction in gross oxygen production under higher nutrient concentration, which had no effect on respiration, yielding significantly lower net photosynthetic rates in all treatments compared to control (Fig. 3b; Table 1). Furthermore, in contrast to species' metabolic responses to treatment factors, the response in light calcification was similar in both species, with a significant reduction (40–55%) at higher temperature, whereas there was no significant effect of nutrient concentration or an interactive effect between the two factors (Fig. 3c, d; Table 1). However, the higher calcification rates under high-temperature and low nutrient conditions in *L. crispatum* indicated an additive effect of the slight increase due to increased nutrient availability and the decline due to high temperature (Fig. 3c).

3.2. Nitrogen uptake and storage

In both species, nitrate reductase activity (NRA) decreased significantly about 50–60% in response to nutrient addition (Fig. 4a, b;

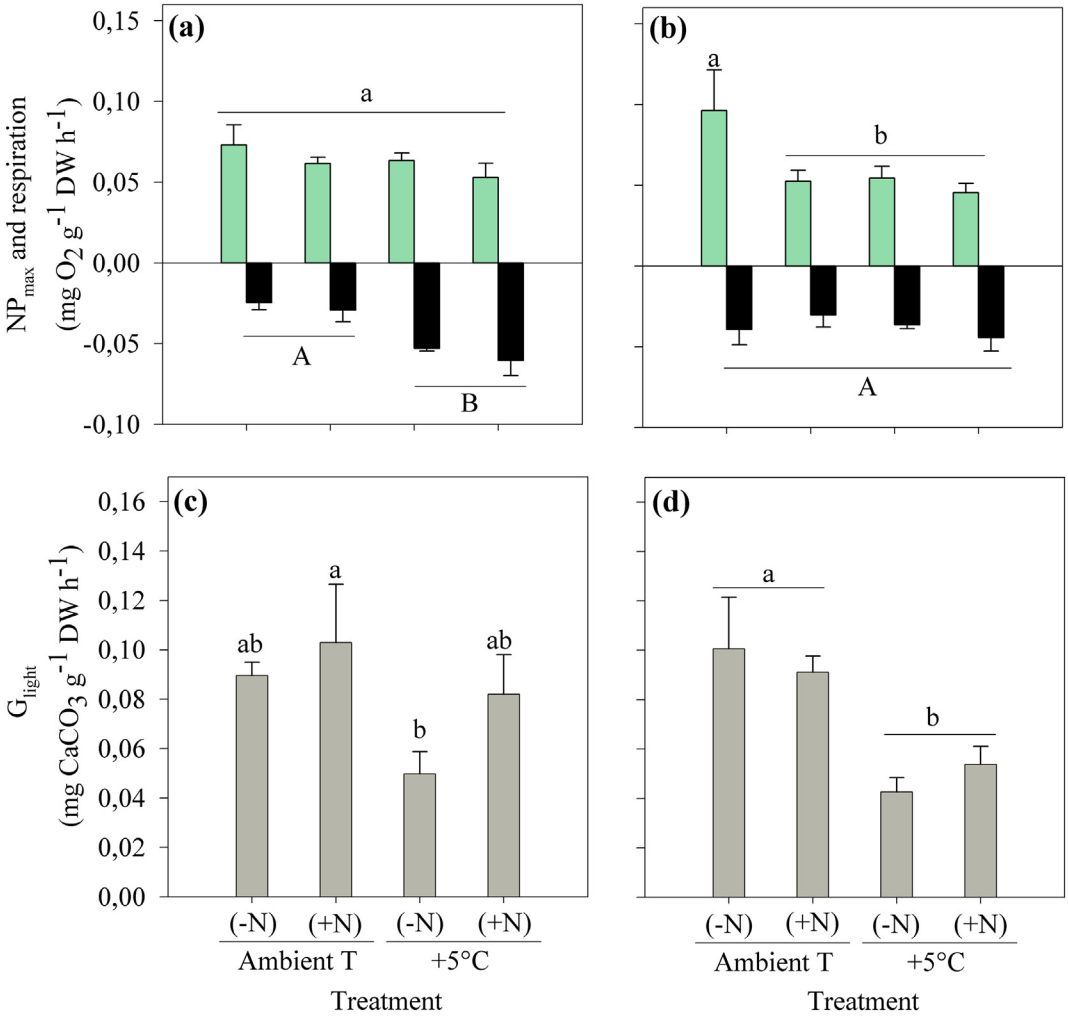

Fig. 3. Maximum net photosynthesis (NP_{max}, dark green bars) (a, b), respiration (black bars) (a, b) and light calcification-G_{light} (c, d) of *L. crispatum* (a, c) and *M. erubescens* (b, d) after exposure for 30 days to different temperature and nutrient conditions (−N: without nutrient addition, +N: with nutrient addition). Data represent mean ± SE and different letters indicate significant differences between treatments (ANOVA, Newman-Keuls posthoc).

Table 1). However, while NRA in *L. crispatum* responded significantly only to nutrient availability, in *M. erubescens* it also decreased under high-temperature, but low-nutrient conditions (Fig. 4b).

The nitrogen content in the algal tissue exhibited an inverse pattern to NRA in *L. crispatum*, with significantly higher values under higher nutrient availability (Table 1). Here, a 60% increase of nitrogen was found under nutrient enrichment, which was lower (43%) when combined with high temperature (Fig. 4c). The same was not found in *M. erubescens*, where higher temperature and/or nutrient availability resulted in significantly lower nitrogen content in the tissue (39–48% lower) and hence, similar values in three of the four treatments (Fig. 4d).

The above effects on tissue nitrogen, together with the similar carbon content in all treatments, led to significantly lower carbon to nitrogen ratios (C:N) under high nutrient concentration in *L. crispatum*. In contrast, in *M. erubescens* nutrient concentration did not affect C:N, while temperature had a significant effect, resulting in higher C:N at higher temperature (Tables 1, 2).

3.3. Pigments

Species-specific differences were also found in the pigment response to the different treatments. In *L. crispatum*, phycobilipigment (PBP) to Chl*a* ratio was reduced significantly due to higher nutrient concentration and temperature, while Chl*a* and PBP content and the ratio of phycoerythrin to phycocyanin (PE:PC) did not respond to the different treatments (Tables 1, 2). *Melyvonnea erubescens* did not exhibit significant effects to either temperature or nutrient concentration on Chl*a* and phycobilipigment content, but PE:PC was significantly reduced under higher temperature (Tables 1, 2).

3.4. Impacts of temperature and nutrients on rhodolith bed primary and carbonate production

The daily integration of *L. crispatum* and *M. erubescens* productivity and their responses to temperature and nutrient availability were significantly affected by both treatment factors (Table 3), and no differences were found between the two species. Net productivity declined significantly in response to either increased nutrient concentration or temperature, resulting in a 60–70% decrease under high-nutrient conditions, but an 80–100% reduction in the two species under high temperature (Fig. 5a). In addition, even though no interaction between the two factors was found, their combined negative impacts were additive, resulting in negative net carbon release rather than carbon assimilation in both species (Fig. 5a), thus potentially transforming the rhodolith bed from a carbon sink to a carbon source.

In contrast to the similar net primary productivity of the two species, they differed significantly in their daily integrated carbonate production

Table 1
Summary of results of the two-way ANOVA analysis for the different response parameters of two rhodolith species (n = 4 per treatment; significant effects are highlighted in bold).

Response parameter	Temperature		Nutrients		Temperature × Nutrients	
	F	p-Value	F	p-Value	F	p-Value
L. crispatum						
GP_{max}	6.26	**0.0294**	0.37	0.5558	0.06	0.8044
NP_{max}	1.44	0.2558	2.04	0.1813	0.01	0.9408
Respiration	23.9	**0.0005**	0.95	0.3491	0.06	0.8095
G_{light}	7.39	**0.020**	4.14	0.0667	0.71	0.4162
Chl*a*	0.11	0.7509	1.57	0.2423	2.00	0.1905
PBP	1.62	0.2300	0.018	0.8972	0.28	0.6067
PBP:Chl*a*	3.31	0.0987	0.60	0.4565	6.39	**0.0299**
PE:PC	0.55	0.4739	1.81	0.2051	0.008	0.9319
NRA	0.75	0.4049	19.7	**0.0010**	0.001	0.9917
Carbon content	0.18	0.6801	0.51	0.4896	0.12	0.7336
Nitrogen content	0.005	0.9474	13.9	**0.0047**	2.36	0.1592
C:N	0.09	0.7702	11.9	**0.0073**	1.95	0.1952
M. erubescens						
GP_{max}	2.45	0.1482	4.97	**0.0499**	4.59	0.0578
NP_{max}	5.65	**0.0388**	6.65	**0.0275**	2.87	0.1213
Respiration	0.76	0.4031	0.004	0.9510	1.79	0.2105
G_{light}	19.6	**0.0013**	0.006	0.9400	0.93	0.3588
Chl*a*	0.08	0.7843	0.49	0.4998	1.34	0.2746
PBP	4.22	0.0644	0.30	0.5926	0.16	0.6934
PBP:Chl*a*	2.07	0.1838	0.95	0.3562	1.48	0.2550
PE:PC	5.78	**0.0350**	0.07	0.8009	0.17	0.6826
NRA	3.44	0.0885	49.7	<**0.0001**	4.62	0.0528
Carbon content	0.52	0.4828	0.24	0.6315	0.69	0.4221
Nitrogen content	11.7	**0.0065**	5.72	**0.0378**	5.43	**0.0419**
C:N	6.04	**0.0337**	3.35	0.0967	0.86	0.3750

GP_{max} and NP_{max} - maximum gross and net photosynthesis, G_{light} - light calcification, PBP - phycobilipigments, PE:PC - phycoerythrin to phycocyanin ratio, C:N - carbon to nitrogen ratio.

due to differences in dark calcification rates, resulting in 40–65% lower $CaCO_3$ production of *M. erubescens*, compared to *L. crispatum* (Fig. 5b; Table 3). The within-treatment response followed the pattern of light calcification, with a significant decrease in carbonate production under higher temperature (Fig. 5b).

When the respective species' biomass per m^2 in the rhodolith bed at Arvoredo Island was considered, they achieved similar daily productivity of 2.8 and 3.1 mg C m^{-2} for *L. crispatum* and *M. erubescens*, respectively (ANOVA, p = 0.8464). This was not surprising, as the species exhibited similar net primary productivity and comparable biomass in the bed (4.7 and 4.9 kg m^{-2} bed for *L. crispatum* and *M. erubescens*, respectively; Carvalho, 2018). In contrast, due to the aforementioned differences in dark calcification rates, *L. crispatum* and *M. erubescens* differed significantly in their daily carbonate production per m^2 (ANOVA, p = 0.0492), with the former producing 35% more $CaCO_3$ m^{-2} rhodolith bed day^{-1}, compared to *M. erubescens*.

In addition, when extrapolating the estimated rhodolith net productivity and carbonate production to the entire rhodolith bed in the Marine Biological Reserve of Arvoredo (100 km^2), the photosynthetic carbon assimilation of the bed was estimated at 579 kg day^{-1}, with a carbonate production of 997 kg day^{-1}. Based on the species' experimental responses to different nutrient concentration and temperature scenarios, under high-nutrient conditions the rhodolith bed productivity may potentially decline by almost 50%, while carbonate production would not be affected (Fig. 5c). In the case of a heat wave event, where temperature can increase dramatically over a time span of a few days, the rhodolith bed primary productivity would potentially experience a severe impact, assimilating 95% less C per day, while productivity would be lower than daily respiration when this event occurs under high-nutrient conditions (Fig. 5c). In the latter case, the rhodolith bed would release more C through respiration than fixing it through photosynthesis. Regarding rhodolith carbonate production, it would potentially be reduced by 24–50% in the case of a heat wave event, with the magnitude depending on nutrient availability (Fig. 5c).

4. Discussion

This study shows that the physiological responses of rhodoliths to a rapid increase in temperature, such as what is experienced during heat wave events, and to increased nutrient concentrations, are species-specific. This type of unique response appears to be a common pattern in coralline algae, as indicated by the wide array of responses to temperature increase (reviewed in Martin and Hall-Spencer, 2017) and nutrient enrichment (Table 4). Thus, the magnitude of the impact of these conditions on the productivity and carbonate production of coastal rhodolith beds are likely to differ considerably depending on the community structure that normally presents particular dominance level of one or few species.

4.1. Rhodolith performance under different temperature and nutrient conditions

The photosynthetic response of the studied rhodolith species differed, as *L. crispatum* did not respond to changes in nutrient concentration but up-regulated both gross photosynthesis and respiration at higher temperature, resulting in similar net photosynthesis in all treatments. On the other hand, net photosynthesis was strongly reduced in *M. erubescens* under increased temperature and/or nutrient concentration. In contrast, the calcification process in both species responded similarly to the 5 °C-temperature rise; both experienced a strong decrease in calcification rates, without being affected by nutrients, a response also found previously in other coralline algae in laboratory and in situ nutrient enrichment studies (Table 4).

Previous studies on the effects of increased temperature in coralline algae have shown that the type of response will depend on the magnitude of the increase. A small rise in temperature which is within the species natural range can increase growth, photosynthesis and calcification, while increases above the thermal maximum of the species' natural habitat cause adverse effects, in both tropical and temperate coralline algae (Ichiki et al., 2001; Blake and Maggs, 2003; Steller et al., 2007; Guenther, 2016; Vásquez-Elizondo and Enríquez, 2016; Vogel et al., 2016; Tanaka et al., 2017; Graba-Landry et al., 2018). The recorded maximum summer water temperature at the collection site varies between 28 and 29 °C (Fig. 1d), and thus, the high-temperature treatment (28 °C), used in this study, was still within the species' natural thermal range. Both studied species are considered cosmopolitan and are found in the Mediterranean, the Atlantic, Pacific and Indian Oceans (Riosmena-Rodríguez et al., 2017). They also have a wide latitudinal range along the Brazilian coast, from the subtropical to the tropical regions (Amado-Filho et al., 2017), all of which indicates that they should be able to tolerate and thrive under temperatures as high as or even higher than those used in this study. Indeed, *L. crispatum* seemed to adjust its metabolism by increasing gross photosynthesis to counterbalance the increased respiration, while *M. erubescens* was not able to do the same, suffering a significant decline in net photosynthesis. Despite these species-specific differences in photosynthetic acclimation to changing thermal conditions, both species experienced a similar strong decline in calcification at 28 °C. This discrepancy points towards species' acclimation/adaptation to local environmental conditions and a somehow limited physiological plasticity to respond to sudden environmental changes, such as heat waves, where temperature rises by several degrees within a short time span (e.g., a 4 °C-rise within a few days recorded in the region, Gouvêa et al., 2017).

High-nutrient conditions had little effect on photosynthesis and calcification of *L. crispatum*, while significantly affecting the photosynthetic performance of *M. erubescens* (Fig. 3). Mixed responses of coralline algal metabolism, growth and abundance to nutrient enrichment have been

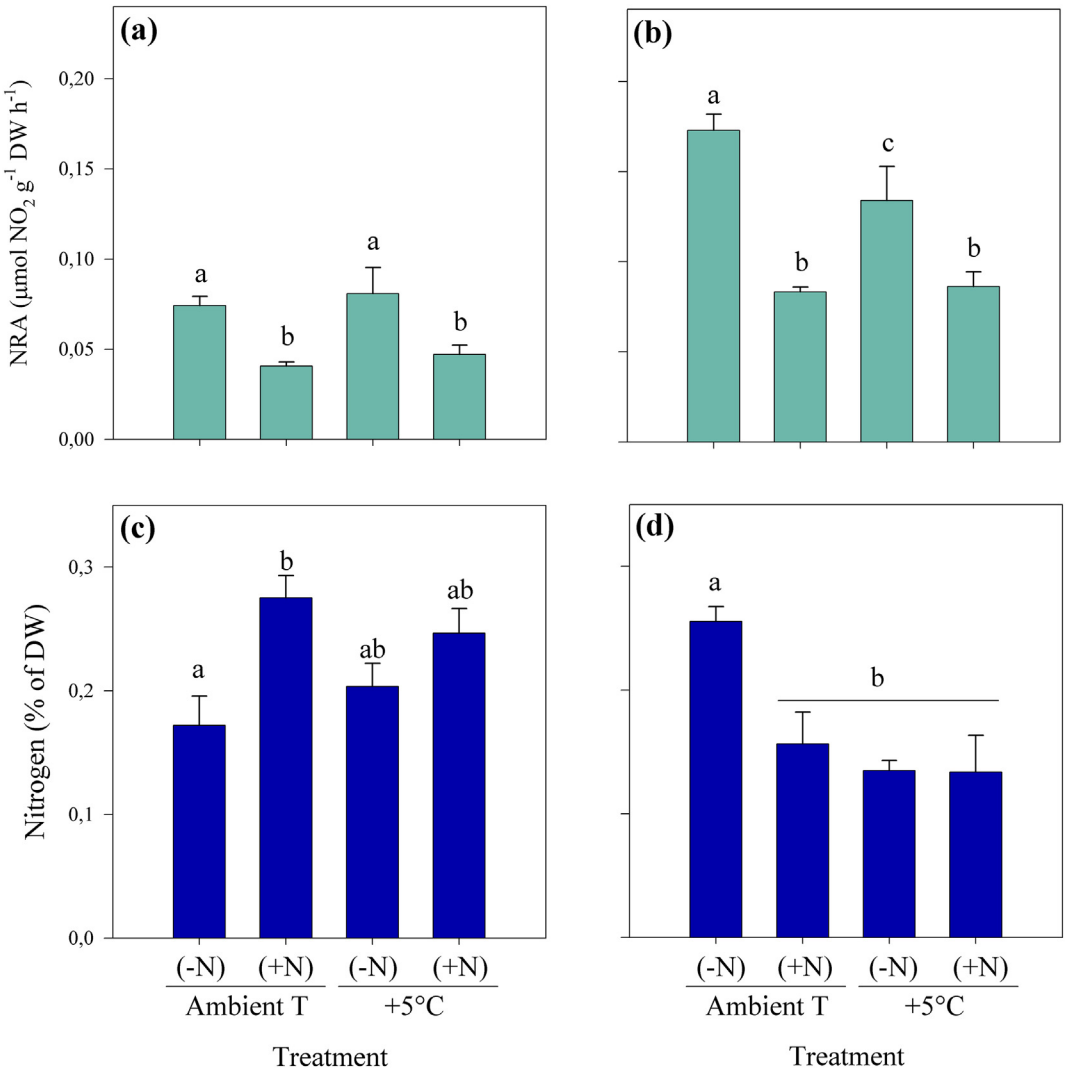

Fig. 4. Nitrogen uptake and storage of *L. crispatum* (a, c) and *M. erubescens* (b, d) in response to different temperature and nutrient conditions (−N: without nutrient addition, +N: with nutrient addition): (a, b) Nitrate reductase activity and (c, d) tissue nitrogen content. Data represent mean ± SE and different letters indicate significant differences between treatments (ANOVA, Newman-Keuls posthoc).

reported, encompassing negative, positive and neutral effects (Table 4). Similar to our results, nutrient enrichment has been found to have no effect on calcification in several coralline algal species (Table 4). Nevertheless, and contrary to our findings, some studies have reported increased net photosynthesis in response to higher nutrient availability and only one reported a decrease. Yet, identifying a general pattern for coralline algal response to nutrient enrichment by comparing studies and species is difficult due to (a) the low number of studies available and (b) the differences in experimental treatments. In fact, testing for individual and/or combined nutrient effects and using different nutrient concentrations leads to highly variable responses, even for the same species (Table 4).

Table 2
Tissue carbon and nitrogen content (% of DW), carbon to nitrogen ratio (C:N), and pigment concentrations (in µg g^{-1} FW) in two rhodolith species exposed to different temperature and nutrient conditions (−N: without nutrient addition, +N: with nutrient addition) for 30 days. Data represent mean ± SE (n = 4 per treatment) and significant differences between treatments for the respective species and parameter are indicated by different letters (two-way ANOVA, Newman-Keuls).

Treatment	Carbon	Nitrogen	C:N	Chl*a*	PBP	PBP:Chl*a*	PE:PC
L. crispatum							
Ambient T (−N)	13.1 ± 0.2^{a}	0.17 ± 0.02^{a}	77.8 ± 10.8^{a}	32.0 ± 0.7^{a}	907 ± 92^{a}	25.4 ± 1.5^{a}	10.4 ± 1.5^{a}
Ambient T (+N)	13.2 ± 0.3^{a}	0.28 ± 0.02^{b}	48.3 ± 2.3^{b}	44.3 ± 1.5^{a}	848 ± 65^{a}	20.9 ± 1.5^{ab}	13.4 ± 1.4^{a}
+5 °C (−N)	13.1 ± 0.5^{a}	0.20 ± 0.02^{ab}	67.5 ± 6.2^{ab}	37.0 ± 8^{a}	746 ± 132^{a}	20.7 ± 1.1^{b}	12.0 ± 1.1^{a}
+5 °C (+N)	13.4 ± 0.4^{a}	0.25 ± 0.02^{ab}	55.0 ± 3.4^{ab}	36.3 ± 1.9^{a}	782 ± 53^{a}	21.6 ± 0.8^{ab}	15.3 ± 4.5^{a}
M. erubescens							
Ambient T (−N)	13.3 ± 0.2^{a}	0.26 ± 0.01^{a}	52.9 ± 2.4^{a}	23.0 ± 3.7^{a}	467 ± 80^{a}	20.1 ± 0.7^{a}	14.7 ± 1.4^{a}
Ambient T (+N)	13.2 ± 0.1^{a}	0.16 ± 0.03^{b}	89.3 ± 13.4^{a}	17.1 ± 2.0^{a}	409 ± 66^{a}	27.5 ± 1.2^{a}	15.8 ± 2.6^{a}
+5 °C (−N)	13.0 ± 0.1^{a}	0.13 ± 0.01^{b}	97.5 ± 6.1^{a}	15.8 ± 2.9^{a}	318 ± 34^{a}	19.3 ± 4.1^{a}	11.7 ± 1.3^{a}
+5 °C (+N)	13.2 ± 0.2^{a}	0.13 ± 0.03^{b}	109.5 ± 21.3^{a}	19.9 ± 2.9^{a}	309 ± 51^{a}	18.5 ± 3.8^{a}	11.5 ± 1.3^{a}

PBP - phycobilipigments, PE:PC - phycoerythrin to phycocyanin ratio.

Table 3
Summary of results of the three-way ANOVA analysis for species-specific responses to different temperatures and nutrient concentrations in the daily integrated photosynthetic carbon assimilation and carbonate production of two rhodolith species (significant effects are highlighted in bold).

Factor	Net C assimilation ($mg\ g^{-1}\ DW\ d^{-1}$)		$CaCO_3$ production	
	F	p-Value	F	p-Value
Species	0.27	0.6107	39.4	<**0.0001**
Temperature	18.9	**0.0003**	19.2	**0.0003**
Nutrients	6.11	**0.0221**	3.19	0.0887
Species × Temp.	0.75	0.3964	0.09	0.7708
Species × Nutrients	0.17	0.6819	2.94	0.1013
Temp. × Nutrients	0.09	0.7699	1.37	0.2549
Species × Temp. × Nutrients	0.19	0.6619	0.08	0.7800

The lack of a positive response of photosynthesis to higher nutrient availability suggests that the studied rhodoliths may not be nutrient-limited under ambient conditions (see Turpin, 1991; Littler and Littler, 1992), likely due to the generally low nutrient demands of these organisms, as recently suggested by McConnico et al. (2018). This assumption is supported by several of our findings. Firstly, the inverse relationship between NRA (indicative for the amount of the enzyme) and nutrient concentration found in both species, when usually nutrient addition induces an increase in NRA under nutrient-limited conditions (e.g., Maier and Pregnall, 1990; Corzo and Niell, 1994; Young et al., 2009). Instead, the high nitrate concentration seemed to have decreased the need for synthesizing nitrate-reductase enzymes, thus lowering energetic expenses, as enzyme synthesis is a costly process, or may have induced the inactive form of the enzyme (Rigano et al., 1981). This resulted in higher tissue nitrogen content in *L. crispatum* when nutrient concentration was high, even though NRA was lower (Fig. 4a, c). Thus, the tissue nitrogen content in this species, which was in the same range as found in other subtropical and temperate rhodoliths (0.13–0.27%, McConnico et al., 2018, Qui-Minet et al., 2018), followed the same pattern as the nitrogen concentration in the seawater, which also was recently reported for the temperate *Lithothamnion corallioides* (Qui-Minet et al., 2018). Secondly, the similar carbon tissue content in all treatments was

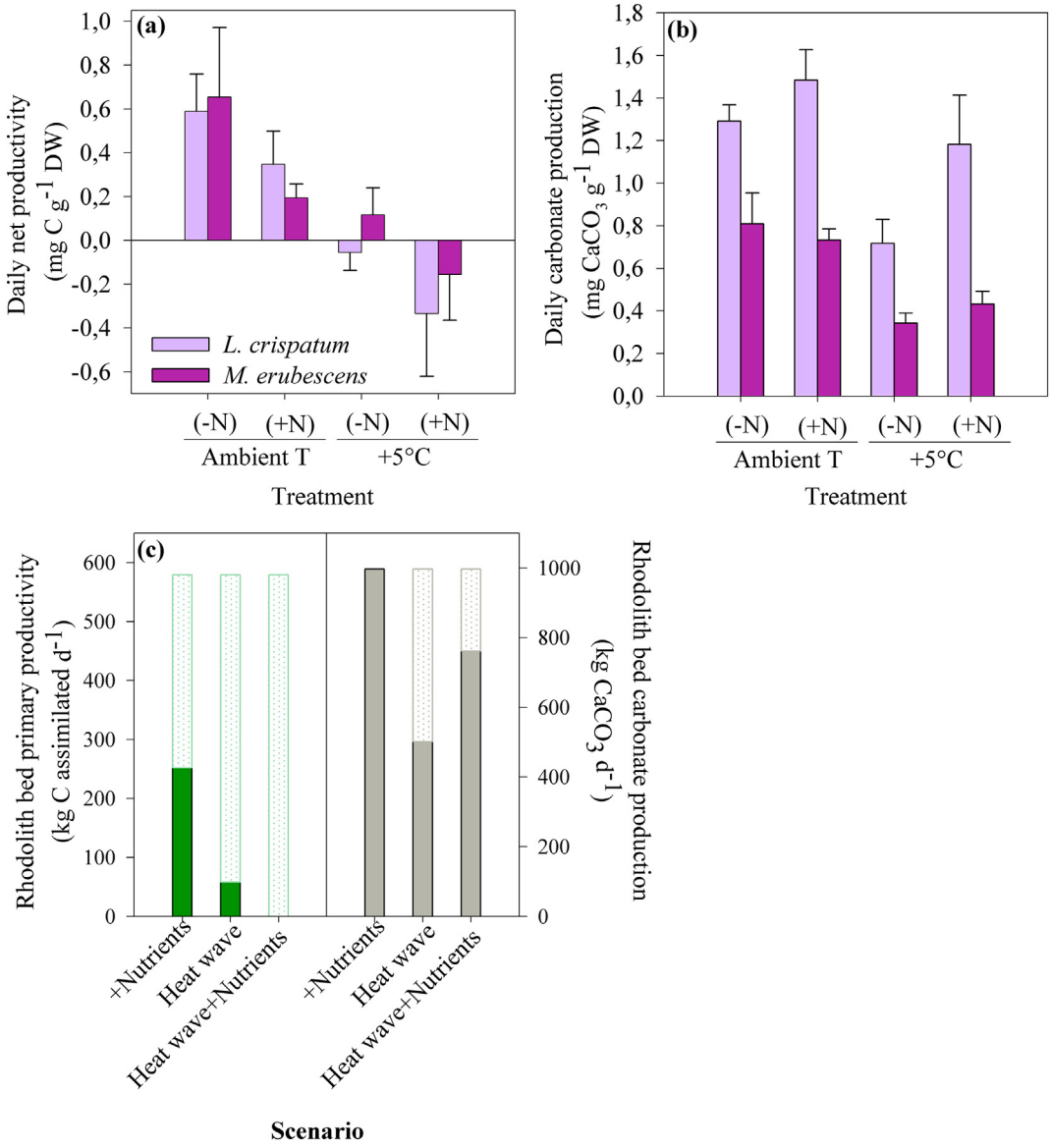

Fig. 5. Rhodolith bed primary productivity and estimated impacts under different scenarios. (a) Daily net primary and (b) carbonate production of rhodolith species under different temperature and nutrient conditions (−N: without nutrient addition, +N: with nutrient addition). (c) Potential impacts of different scenarios (increased nutrient input, heat wave) on the primary and carbonate production of the entire rhodolith bed at Arvoredo Island (filled stacks-estimated primary and carbonate production under a given scenario, dotted stacks-potential loss). Data represent mean ± SE and different letters indicate significant differences between treatments (ANOVA, Newman-Keuls posthoc).

Table 4
Summary of reported effects of nutrient enrichment in coralline algae (* - in situ enrichment; lower nutrient concentration represents control value and higher concentration the nutrient-enriched treatment; NP - net photosynthesis, R - respiration, G_{net} and G_{light} - net and light calcification, PBP - phycobilipigments).

Species	Locality	Nutrients	Nutrient concentration (μM)	Biological response	Reference
Lithophyllum sp.	South Australia	Nitrate + Nitrite	0.3–4.9	↓ Biomass	Russell et al. (2009)
		Ammonium	0.4–1.1	No effect on cover and F_v/F_m	
		Phosphate	0.7–1.1		
Lithophyllum kotschyanum	French Polynesia	Nitrate	0.55–5.5	N, P: ↑ [Chl*a*], [PBP], no effect on R, NP and G_{net}	Shayka (2018)
		Phosphate	0.17–1.7	N + P: ↑ NP, R G_{net}, [Chl*a*] and [PBP]	
	Tanzania	Nitrate	0.7–10	P(>1 μM): ↓ Growth and G_{light}	Björk et al. (1995)
		Ammonium	0.7–10	N: no effects	
		Phosphate	0.7–10		
	*Great Barrier Reef	Nitrate	0.65–36	No effect on G	Steven (2000)
		Phosphate	0.2–5.1		
	*Great Barrier Reef	Ammonium	1.3–20 μM	No effect on growth, calcification	Koop et al. (2001)
		Phosphate	0.2–4 μM		
Lithophyllum pygmaeum	Great Barrier Reef	Ammonium	1.4–8	↓ Growth, NP	Lewis (2016)
		Phosphate	0.1–1.6		
Lithophyllum yessoense	Japan	Nitrate	0.5–25	No effect on growth	Ichiki et al. (2000)
		Phosphate	0.5–5		
Porolithon onkodes	Japan	Phosphate	0.1–2	↑ NP, no effect on G_{net} and [Chl*a*]	Tanaka et al. (2017)
	French Polynesia	Nitrate + Nitrite	0.7–1	↑ G_{net}, [Chl*a*], [PBP]	Johnson and Carpenter (2018)
		Ammonium	6.3–19.		
Corallina elongata	Spain	Nitrate	0–10	↑ [PBP], tissue N	Vergara and Niell (1993)
Corallina officinalis	South Australia	Phosphate	7.5–30	↓ Growth	Brown et al. (1977)
Cheilosporum sagittatum			3.8–30		
Jania rubens			7.5–30		

contrary to what is normally found in nutrient-limited algae, where starch accumulates to be later degraded and used as a source for amino acid synthesis when nutrients become available (Elrifi and Turpin, 1986; Macler, 1986; Smith et al., 1989). Finally, the lack of changes in pigment content among treatments indicates no nutrient limitation. Red algae can use pigments (Chl*a*, phycobilipigments) as N reservoir and thus their concentration often follows the pattern of nutrient availability, a feature also commonly observed in other coralline algae (Table 4). However, even though no significant changes in chlorophyll and phycobilipigments were found, their contents still tended to correlate positively with tissue nitrogen, similar to previous findings in other red algae (Lapointe, 1981).

As discussed above, *L. crispatum* showed an inverse pattern of NRA and tissue nitrogen, with the latter following the nitrogen availability in the water. However, while a relatively similar response for NRA was found in *M. erubescens*, in this species NRA also decreased when temperature was higher (Fig. 4b). The different response of this species was also reflected in the tissue nitrogen content, as higher nutrient concentration did not result in higher tissue N in *M. erubescens*, but in a significant reduction, a response also found under exposure to high temperature (Fig. 4d). This might be related to the similar response pattern found in gross photosynthesis, as this process is coupled with nitrogen uptake and a decline in the former may have resulted in less energy available for nitrogen assimilation (Turpin, 1991; Huppe and Turpin, 1994). In turn, ammonium assimilation has been shown to cause a decrease in gross oxygen evolution (Elrifi and Turpin, 1986; Turpin et al., 1988), which seems unlikely in our study, as it is usually coupled with an increase in respiratory rates, a response we did not find in *M. erubescens*. A possible explanation for this might be a direct effect of nutrients on the rhodolith holobiont rather than on the algae itself, as increased nutrient availability and/or temperature might have caused shifts in the rhodolith-associated bacterial community, causing an indirect effect on rhodolith photosynthetic performance and nitrogen uptake. In this context, a wide range of beneficial and detrimental interactions have been described between macroalgae and their microbiomes, based on the exchange of nutrients, minerals and secondary metabolites (Hollants et al., 2012). Recently, it has been shown that rhodolith-associated bacterial communities are enriched in aerobic ammonia-oxidizing beta-proteobacteria and dissimilative sulfate-reducing delta-proteobacteria (Cavalcanti et al., 2014, 2018), and another study suggested that associated bacteria might play a role in nutrient cycling (McConnico et al., 2018). Yet, so far no information is available about potential functions of associated bacterial communities among rhodoliths that might give some indications about possible differences between the two studied species. Also, unlike with the effects of ocean acidification (Cavalcanti et al., 2018), to date there are no studies on how rhodolith bacterial community responds to environmental changes related to temperature or nutrient enrichment. However, temperature related changes in coralline-algal-associated bacterial communities have been reported for the temperate articulated *Amphiroa gracilis* and the tropical crustose *Neogoniolithon fosliei* (Webster et al., 2011; Huggett et al., 2018). Thus, besides direct temperature and nutrient effects on rhodolith performance, we cannot exclude that the treatment conditions caused a concomitant disturbance of the rhodolith-associated microbiome, translating to indirect effects on performance, as also recently suggested by Cavalcanti et al. (2018).

4.2. Impacts of temperature and nutrients on rhodolith bed primary and carbonate production

The daily net productivity of the studied subtropical rhodolith bed at Arvoredo Island ranged between reported estimates for a tropical bed composed of *Lithophyllum kotchyanum* and the lower productivity reported for temperate beds, composed mainly of *Lithothamnion corallioides* (Table 5). Unfortunately, there are no reports of carbonate production rates of tropical rhodolith beds, but the rates estimated here were found to be comparable with estimates for a shallow temperate rhodolith bed during summer and those estimated for tropical crustose coralline algae (Table 5). However, our study also indicates that carbonate production is dependent on the rhodolith species that dominates the bed, as the two species studied here differed significantly in their daily production rate (Fig. 5b). The latter was related to strong differences in their dark rather than light calcification rates: While *L. crispatum* calcifies in the dark at a rate of 20% of light calcification, *M. erubescens* decalcifies at night. These species-specific differences agree with the wide variability of dark calcification rates reported for coralline algae and also specifically for rhodoliths. In rhodoliths, net calcification in darkness has been reported to range between 8 and 63% of light calcification rates (King and Schramm, 1982; Martin et al., 2006; Kamenos et al., 2013; Noisette et al., 2013a; Legrand et al., 2017;

Table 5
Summary of reported daily primary and carbonate production of coralline algae per m^2 of seabed (from single species incubations, * in situ measurements on community; Sp - spring, S - summer, A - autumn, W - winter).

Species	Latitude	Depth	Season	Net productivity (g C m^{-2} d^{-1})	Carbonate production (g $CaCO_3$ m^{-2} d^{-1})	Reference
Free-living						
Lithothamnion corallioides +	48°N	0.3–8	Sp	0.38		Martin et al. (2005)
*Phymatolithon calcareum**		0.5–3	S	−0.64	2.72	Martin et al. (2007)
			W	−0.46	0.05	
Lithothmanion corallioides		1		1.23	7.26	Martin et al. (2006)
		5	S	1.28	5.35	
		10		−1.67	2.30	
		1		0.99	2.55	
		5	W	0.02	1.25	
		10		−0.46	0.65	
Lithothamnion crispatum	27°S	8	A	2.75	6.02	This study
Melyvonnea erubescens				3.05	3.95	
Lithothamnion kotchyanum	23°S	3–7	–	6–14		Koop et al. (2001)
Crustose						
Lithophyllum cabiochae	43°N	25	Sp	0.62	2.3	Martin et al. (2013)
Various species	14°S	0–18	–	0.9–5	0.8–9.1	Chisholm (2000, 2003)
Articulated						
*Ellisolandia elongata**	43°N	3	W	0.88	0.8	Bensoussan and Gattuso (2007)
Ellisolandia elongata	43°N	5	–	2.5–10	13.8	El Haïkali et al. (2004)
	32°N	–	W	3.5	20.8	Guy-Haim et al. (2016)

Schoenrock et al., 2018) and net dissolution (−7% of light calcification rate; Sordo et al., 2018). This seems to be a common pattern in corallines, as similar findings have been reported by Chisholm (2000), when comparing different crustose coralline species. While some species decalcify in the absence of light (−4 to −23% of light calcification), others still exhibit a positive net calcification (7.5–15% of light calcification). Thus, it can be assumed that rhodolith bed carbonate production will vary according to which species dominates the community. Furthermore, the different responses found between the two studied rhodolith species (*L. crispatum*'s metabolism responding strongly only to temperature while *M. erubescens* was affected also by eutrophic conditions) and the differences in carbonate production will have strong implications in future scenarios for the rhodolith bed community. The impact of either temperature or eutrophic conditions will be more or less severe, depending on the dominance of one species or the other within the community.

Overall, in the context of ongoing climate change and related phenomena, our results suggest that rhodolith bed primary and carbonate production will potentially decline in regions impacted by increased nutrient input and/or heat wave events (Fig. 5c), whose intensity and frequency has already increased and will continue do so in the near future (Meehl and Tebaldi, 2004; Gouvêa et al., 2017; Frölicher et al., 2018; Oliver et al., 2018). Also, while temperature and nutrients apparently do not interact, their negative impacts on daily rhodolith bed primary production are additive (Fig. 5a), indicating that heat wave events will impact rhodolith beds more severely in coastal regions with increased nutrient input. This is especially worrisome, as in recent years this region of the Brazilian coast has been subject to several marine heat waves, and there is already experimental evidence on its impacts on the performance of fleshy macroalgae (Gouvêa et al., 2017). Thus, increased nutrient concentrations due to upwelling events (influenced by the Prata River discharge) and urban run-offs (especially during summer) could cause a combined effect on the region's coastal habitats, and the rhodolith bed of the Arvoredo Marine Biological Reserve could be particularly vulnerable. This in turn will have implications for the rhodolith bed-associated ecosystem services, as these communities constitute areas of great interest for $CaCO_3$ exploitation that supplies agricultural and industrial applications (Amado-Filho and Pereira-Filho, 2012, Moura et al., 2013), provide nursery grounds and habitat for numerous commercial species of invertebrates and fishes (Simon et al., 2016), and are important for the carbon and carbonate budget of these shallow coastal ecosystem.

Rhodolith bed communities represent major contributors to coastal CO_2 fluxes through high community photosynthesis and respiration and $CaCO_3$ production and dissolution (Martin et al., 2005, 2006, 2007; Basso, 2012). Therefore, environmental impacts on these communities can substantially alter the carbonate chemistry of the water column and the ability of the oceans to take up atmospheric CO_2 (Andersson et al., 2005). Regarding local management strategies, our findings provide useful information, as they show that reducing local stressors (e.g. nutrient pollution) may reduce the effects of global stressors not covered by local governance (e.g. ocean warming) on rhodolith beds.

5. Considerations and future research directions

Rhodolith beds cover extensive areas along the Atlantic coasts (Foster, 2001; Riosmena-Rodríguez et al., 2017), assimilating/releasing carbon through photosynthesis and respiration, respectively, and storing massive amounts of $CaCO_3$. Yet, our knowledge regarding their importance in the global carbon cycle is still extremely scarce (Basso, 2012). The little available information is mainly focused on temperate European rhodolith beds, while information on productivity and responses to environmental changes on Brazilian rhodolith species and communities is still missing, despite Brazil's coast harbouring the most extensive rhodolith beds worldwide.

In this study, we present the very first estimates of community metabolism and carbonate production for a subtropical (Brazilian) rhodolith bed. Being an important first step to gain insight of the importance of rhodolith beds for coastal carbon fluxes, it should be pointed out that these measurements are derived from incubations of individual rhodoliths. Rhodolith beds provide structurally complex habitats, colonized by a large diversity of both autotrophic and heterotrophic organisms, some of which are also calcifiers (e.g., Barbera et al., 2003; Dias and Villaça, 2012). Thus, a comprehensive evaluation of the carbonate production of rhodolith communities should take into account this associated fauna and flora and its roles, as well as other processes (e.g., sloughing, bioerosion). The same applies for rhodolith bed net primary productivity, as associated heterotrophic epi- and infauna can contribute significantly to community respiration, causing temperate beds to be a net carbon source, rather than a sink, over a diel cycle,

despite substantial primary production (e.g., Martin et al., 2007; Attard et al., 2015). On the other hand, high biomass of associated flora might add substantially to gross rhodolith bed productivity. For these reasons primary and carbonate production determined from entire communities through in situ incubations has shown lower values than those based on estimates derived from single rhodolith incubations (see Table 5). In addition, there is strong seasonal variation, influencing metabolism and carbonate production (Martin et al., 2006, 2007; Legrand et al., 2017), as well as community composition (Amado-Filho et al., 2007, 2010; Dias and Villaça, 2012; Pascelli et al., 2013; Qui-Minet et al., 2018) and responses to changing environmental conditions, e.g. increased temperature (see Martin and Gattuso, 2009).

Thus, future research should be focused on assessing (1) the importance of rhodolith beds in the global carbon cycle, by intensifying the efforts to determine community productivity and carbonate production, including variations due to season, latitude and community composition, and (2) the responses of rhodoliths and associated fauna and flora to environmental changes, such as those related to ongoing climate change and local stressors, in order to determine their resilience and/or susceptibility and the resulting changes in carbon fluxes (Martin and Gattuso, 2009).

CRediT authorship contribution statement

N. Schubert: Conceptualization, Investigation, Data curation, Writing - original draft, Writing - review & editing. **V.W. Salazar:** Investigation, Data curation. **W.A. Rich:** Investigation, Data curation. **M. Vivanco Bercovich:** Investigation, Data curation. **A.C. Almeida Saá:** Investigation, Data curation. **S.D. Fadigas:** Investigation, Data curation. **J. Silva:** Writing - review & editing, Funding acquisition. **P.A. Horta:** Conceptualization, Writing - review & editing, Funding acquisition.

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